L1: Entry 7 of 7 File: USPT Jan 6, 1998

US-PAT-NO: 5705391
DOCUMENT-IDENTIFIER: US 5705391 A

TITLE: Modified acyl-ACP desaturase

DATE-ISSUED: January 6, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cahoon; Edgar B.	Shoreham	NY		
Shanklin; John	Shoreham	NY		
Lindgvist; Ylva	Jarfalla			SE
Schneider; Gunter	Jarfalla			SE

US-CL-CURRENT: 435/419; 435/189, 435/243, 435/252.3, 435/254.11, 435/255.1, 435/320.1, 536/23.2

CLAIMS:

We claim:

1. A nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at an amino acid contact residue in the substrate binding channel, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

2. The nucleic acid sequence of claim 1 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

3. A DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterize by a point mutation at an amino acid contact residue in the substrate binding channel, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

4. The DNA expression construct of claim 3 wherein the point mutation is introduced into

wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

5. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at an amino acid contact residue in the substrate binding channel, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

6. The cell of claim 5 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid contact residues selected from the group consisting of residues 114, 115, 117, 118, 179, 181, 188 and 189.

7. The cell of claim 5 which is a prokaryotic cell.

8. The cell of claim 5 which is a eukaryotic cell.

9. The cell of claim 8 which is a plant cell.

Previous Doc Next Doc Go to Doc#

L1: Entry 11 of 12 File: USPT Aug 8, 2000

US-PAT-NO: 6100091
DOCUMENT-IDENTIFIER: US 6100091 A

TITLE: Modified acyl-ACP desaturase

DATE-ISSUED: August 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cahoon; Edgar B.	Shoreham	NY		
Shanklin; John	Shoreham	NY		
Lindgvist; Ylva	Jarfalla			SE
Schneider; Gunter	Jarfalla			SE

US-CL-CURRENT: 435/455; 435/189, 435/252.3, 435/254.11, 435/320.1, 435/325, 435/410, 435/440, 536/23.2

CLAIMS:

We claim:

1. A method for modifying the chain length and double bond positional specificities of a soluble plant fatty acid desaturase, the method comprising modifying one or more amino acid residues in the substrates binding channel of the soluble plant fatty acid desaturase which do not make direct contact with substrate.

2. A method for modifying the chainlength and double bond specificities of a soluble plant fatty acid desaturase, the method comprising modifying the amino acid residue corresponding to amino acid 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

3. The method of claim 1 wherein the amino acid residues are located at the upper part of the substrate binding channel of the soluble fatty acid desaturase.

4. The method of claim 3 wherein the soluble plant fatty acid desaturase is an acyl-ACP desaturase.

5. The method of claim 4 wherein the acyl-ACP desaturase is a .DELTA..sup.9 desaturase.

6. The method of claim 5 wherein the .DELTA..sup.9 desaturase is produced by a plant selected from the group consisting of Thunbergia alata or Ricinus communis.

7. The method of claim 6 wherein the amino acid residues are selected from the group consisting of residues corresponding to amino acids 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

8. A method for modifying the chain length and double bond positional specificities of an acyl-ACP desaturase, comprising:

a) providing the primary amino acid sequence of the acyl-ACP desaturase;

b) aligning the primary amino acid sequence of the acyl-ACP desaturase with the primary amino acid sequence of the Ricinus communes .DELTA..sup.9 desaturase for maximum sequence conservation;

c) constructing a 3-dimensional model for the acyl-ACP desaturase based on the sequence conservation with the Ricinus communis .DELTA..sup.9 desaturase;

d) identifying amino acid residues which most closely correspond to amino acids 200, 203, 204, 205, 206, and 207 of the Ricinus communis .DELTA..sup.9 desaturase, of the structure modeled in step c); and

e) generating a mutant acyl-ACP desaturase having modified chain length and double bond positional specificities by replacing one or more of the amino acid residues identified in step d) with another amino acid residue.

9. A mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the mutant containing a point mutation at one or more amino acid residues which do not make direct contact with substrate.

10. The mutant acyl-ACP desaturase of claim 9 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

11. The mutant acyl-ACP desaturase of claim 9 wherein the amino acid residues which do not make direct contact with substrate are selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 desaturase.

12. A mutant acyl-ACP desaturase having one or more amino acid substitutions at residues which do not make direct contact with substrate, which is characterized by changes in chain-length and double bond positional specificity as compared to the wild-type acyl-ACP desaturase counterpart.

13. The mutant acyl-ACP desaturase of claim 12 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the residue corresponds to amino acid 200 of the Ricinus communis .DELTA..sup.9 desaturase.

14. The mutant acyl-ACP desaturase of claim 12 wherein the residues are located at the upper part of the substrate binding channel.

15. The mutant acyl-ACP desaturase of claim 14 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the residues are selected from the group consisting of residues corresponding to amino acids 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.2 ACP desaturase.

16. The mutant of claim 13 or claim 15 wherein the .DELTA..sup.9 acyl-ACP desaturase is produced by mutagenizing nucleic acid cloned from Thunbergia alata or Ricinus communis.

17. A nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid residues which do not make direct contact with

substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

18. The nucleic acid sequence of claim 17 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at the residue corresponding to reside 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

19. The nucleic acid sequence of claim 17 wherein the amino acid residue is located at the upper part of the substrate binding channel of the acyl-ACP desaturase.

20. The nucleic acid sequence of claim 19 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid residues selected from the group consisting of residues corresponding to amino acids 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

21. A DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

22. The DNA expression construct of claim 21 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at the residue corresponding to residue 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

23. The DNA expression construct of claim 21 wherein the amino acid residue is located at the upper part of the substrate binding channel of the acyl-ACP desaturase.

24. The DNA expression construct of claim 23 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid residues selected from the group consisting of residues corresponding to residue 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

25. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

26. The cell of claim 25 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at the amino acid residue corresponding to residue 200 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

27. The cell of claim 25 wherein the amino acid residue is located at the upper part of the substrate binding channel of the acyl-ACP desaturase.

28. The cell of claim 27 wherein the point mutation is introduced into wild-type Ricinus communis .DELTA..sup.9 desaturase at one or more amino acid residues selected from the group consisting of residues corresponding to residue 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

29. The cell of claim 25 which is a prokaryotic cell.

30. The cell of claim 25 which is a eukaryotic cell.

31. The cell of claim 30 which is a plant cell.

32. A DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a chimeric acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold.

33. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises .DELTA..sup.6 -16:0 in which amino acids corresponding to amino acids 172-201 of Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 178-207 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase from a .DELTA..sup.9 -18:0 ACP desaturase.

34. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises .DELTA..sup.6 -16:0 in which amino acids corresponding to amino acids 172-196 of Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 178-202 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase from a .DELTA..sup.9 -18:0 ACP desaturase.

35. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176, 183, 184, 200, 201 and 202 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to 181, 188, 189, 205, 206 and 207 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

36. The DNA expression construct of claim 32 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids 183 and 184 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 188 and 189 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

37. The DNA expression construct of claim 35 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176 and 195 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to 181 and 200 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

38. The DNA expression construct of claim 35 wherein the chimeric acyl-ACP desaturase comprises a .DELTA..sup.6 -16:0 ACP desaturase in which amino acids corresponding to amino acids 176, 195, 200, 201 and 202 of the Thunbergia alata .DELTA..sup.6 -16:0 ACP desaturase are replaced with amino acids corresponding to amino acids 181, 200, 205, 206 and 207 of the Thunbergia alata .DELTA..sup.9 -18:0 ACP desaturase, respectively, from a .DELTA..sup.9 -18:0 ACP desaturase.

39. A method for modifying the chain length and double bond positional specificities of a soluble plant fatty acid desaturase, the method comprising modifying one or more amino acid contact residues in the substrate binding channel of the soluble fatty acid

desaturase which contact the fatty acid, and modifying one or more amino acids which do not contact substrate.

40. The method of claim 39 wherein the soluble plant fatty acid desaturase is an acyl-ACP desaturase.

41. The method of claim 40 wherein the acyl-ACP desaturase is a .DELTA..sup.9 desaturase.

42. The method of claim 41 wherein the .DELTA..sup.9 desaturase is produced by a plant selected from the group consisting of Thunbergia alata or Ricinus communis.

43. The method of claim 42 wherein the amino acid contact residues are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase and the amino acids which do not contact substrate are selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206, and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

44. A method for modifying the chain length and double bond positional specificities of an acyl-ACP desaturase, comprising:

a) providing the primary amino acid sequence of the acyl-ACP desaturase;

b) aligning the primary amino acid sequence of the acyl-ACP desaturase with the primary amino acid sequence of the Ricinus communis .DELTA..sup.9 desaturase for maximum sequence conservation;

c) constructing a 3-dimensional model for the acyl-ACP desaturase based on the sequence conservation with the Ricinus communis .DELTA..sup.9 desaturase;

d) identifying amino acid contact residues within the substrate binding channel of the structure modeled in step c);

e) identifying amino acid residues which most closely correspond to the amino acids 200, 203, 204, 205, 206, and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase of the structure modeled in step c); and

f) generating a mutant acyl-ACP desaturase having modified chain length and double bond positional specificities by replacing one or more of the amino acid contact residues identified in step d) with another amino acid residue, and replacing one or more of the amino acid residues identified in step e) with another amino acid residue.

45. A mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold which contains a point mutation at one or more amino acid contact residues in the substrate binding channel and also contains a point mutation at one or more amino acid residues which do not make direct contact with substrate.

46. The mutant acyl-ACP desaturase of claim 45 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

47. A mutant acyl-ACP desaturase having one or more amino acid substitutions at contact residues within the substrate binding channel and one or more amino acid substations at residues which does not make direct contact with substrate.

48. The mutant acyl-ACP desaturase of claim 47 which is characterized by changes in chain-

length and double bond positional specificity as compared to the wild-type acyl-ACP desaturase counterpart.

49. The mutant acyl-ACP desaturase of claim 48 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the contact residues within the substrate binding channel are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase, and the residue which does not make direct contact with substrate is selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

50. The mutant of claim 49 wherein the .DELTA..sup.9 acyl-ACP desaturase is produced by mutagenizing nucleic acid cloned from Thunbergia alata or Ricinus conmmunis.

51. A nucleic acid sequence encoding a mutant acyl-ACP desaturase characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold which contains a point mutation at one or more amino acid contact residues in the substrate binding channel and further contains a point mutation at one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence alignment with the Ricinus communis .DELTA..sup.9 desaturase.

52. The nucleic acid sequence of claim 51 wherein the first fatty acid has a chain-length of 16:0 and the second fatty acid has a chain-length of 18:0.

53. The nucleic acid sequence of claim 52 wherein the acyl-ACP desaturase is the .DELTA..sup.9 acyl-ACP desaturase and the contact residues within the substrate binding channel are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase, and the residue which does not make direct contact with substrate is selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

54. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence encoding a mutant acyl-ACP desaturase which is characterized by the ability to catalyze desaturation of a first fatty acid and a second fatty acid, the first and second fatty acids differing in their chain length, the desaturation rates of both the first and second fatty acids differing by no more than about 4-fold, the nucleic acid sequence encoding the mutant acyl-ACP desaturase being characterized by a point mutation at one or more amino acid contact residues in the substrate binding channel, and one or more amino acid residues which do not make direct contact with substrate, the nucleic acid sequence being further characterized as having a sufficient degree of amino acid identity with the amino acid sequence of Ricinus communis .DELTA..sup.9 desaturase to enable statistically significant sequence

alignment with the Ricinus communis .DELTA..sup.9 desaturase.

55. The cell of claim 54 wherein the amino acid contact residues are selected from the group consisting of residues corresponding to amino acids 114, 115, 117, 118, 179, 181, 188 and 189 of the Ricinus communis .DELTA..sup.9 ACP desaturase and the amino acid residues which do not make direct contact with substrate are selected from the group consisting of residues corresponding to amino acids 200, 203, 204, 205, 206 and 207 of the Ricinus communis .DELTA..sup.9 ACP desaturase.

56. The cell of claim 54 which is a prokaryotic cell.

57. The cell of claim 54 which is a eukaryotic cell.

58. The cell of claim 57 which is a plant cell.

Previous Doc Next Doc Go to Doc#